

The Chromosome Complex of *Culex Pipiens*.

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With Plates 27 and 28, and 3 Text-figures.

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INTRODUCTION.

MISS STEVENS, in a paper entitled "The Chromosomes in the Germ-Cells of *Culex*," gave as one of her conclusions the following :

"Parasynapsis (parasyndesis¹) occurs in each cell generation of the germ-cells, the homologous maternal and paternal chromosomes being paired in telophase and remaining so until the metaphase of the next mitosis."

Because of the importance of this discovery, especially in the support it lends to the recent theory of parasyndesis, and because of its bearing on the theory of the individuality of the chromosomes, Dr. Agar, in the summer of 1912, at Tay-

¹ I have adopted the word "syndesis" for the conjugation of the chromosomes, and "synizesis" for their clumping together.

vallich, Loch Sween, collected and preserved material in order to investigate the germ-cells of *Culex pipiens*. This he very kindly gave to me, and in the spring and summer of 1913 a supply of egg-rafts, larvæ, and pupæ from Milngavie and Skelmorlie has been used in conjunction with the original stock.

The results obtained by a study of the Tayvallich material showed clearly that a much more extensive investigation than was originally intended would be necessary in order completely to elucidate the problems that incidentally presented themselves. Hence many of the egg-rafts and larvæ were placed in artificial ponds in order that greater control might be exercised over the material to be fixed, and single specimens were isolated so that their exact age could be determined, and their periods of ecdysis watched.

The life-history of *Culex pipiens* is to be found set forth in innumerable text-books of Natural History, and much has been written about mosquitoes in connection with malaria, but in no case have I been able to find any adequate account of the behaviour and fate of the imagines which hatch out and live in captivity, nor of the time that elapses between the emergence of the imago and the deposition of eggs. Large numbers of pupæ, developed, some under natural, others under artificial, conditions, from eggs obtained in May or August of 1913, were placed in small ponds which were covered over with large cages made of mosquito-netting so that the resulting imagines could be observed. From many hundreds of these captive-reared creatures I have not succeeded in obtaining any egg-rafts, although some of the imagines have lived for four months. Nor could they be induced to suck blood, which, according to the account of some naturalists, is necessary, even in non-tropical forms of gnats, for the development of the eggs.

A comparison of the spermathecæ of adults which have always been captive with those of adults taken in the open shows that the former never contain spermatozoa, although the latter do. Hence it would appear that captivity is not

favourable to fertilisation. The completion of this study by the detailed investigation of the maturation and fertilisation of the egg-cell will have to be postponed for an indefinite time until a developmental series of imagines caught in the open has been secured, or until the technique of artificial rearing has been mastered.

Another interesting feature in connection with the larvæ is the want of uniformity in the periods of metamorphosis. Although the main body of any collection of larvæ will complete their development in the usual time, there are always some laggards who double or even treble the usual periods. Temperature and food-supply do not wholly account for this retarded development.

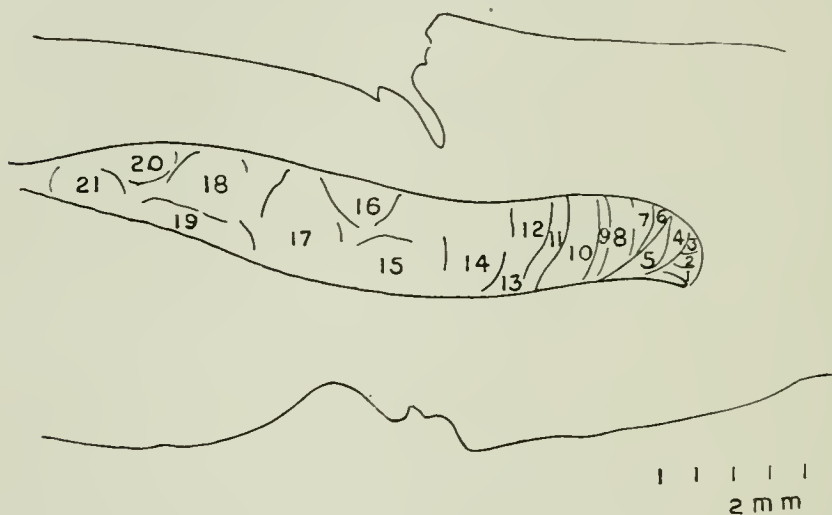
The fixatives employed have been Benda's fluid, acetic bichromate, Gilson's mercurio-nitric, Flemming, and Gilson-Petrunkewitch, the two latter being most successful for the cytology proper; the two former were useful for interpreting cytoplasmic details.

Thionin, iron-hæmatoxylin (prolonged staining), as well as Mayer's cochineal, Ehrlich's hæmatoxylin and safranin were the stains employed. Many slides were first studied in thionin, and then the cover-slip was removed, the thionin washed out, the sections re-stained in iron-hæmatoxylin, and comparisons made between the results of the two stains. For certain stages after prolonged treatment with iron-hæmatoxylin much extraction was necessary, for others little, so that the same slide had often to be studied under various degrees of extraction.

Although aceto-carminé preparations of the whole gonad are very useful for mapping out quickly the main facts of spermatogenesis, and although this stain has the advantage, as Miss Stevens has pointed out, and as my experience has confirmed, of increasing the size of the cellular elements and of thus rendering them easier of examination, they are not permanent, not so good for finer details, and not useful for somatic mitosis. Hence the figures given in this paper have been taken from sections (thickness ranging from $4\ \mu$ to $12\ \mu$) and not from aceto-carminé preparations.

Miss Stevens worked on *Culex pungens*,¹ a form which is very nearly allied to *C. pipiens*. It is probable that this close relationship between *C. pipiens* and *C. pungens* accounts for the great similarity which exists between her figures of primary and secondary spermatocyte anaphases and telophases and those that occur in *C. pipiens*.

TEXT-FIG. 1.



Outline of testis of pupa of *Culex pipiens* to show level at which the various stages in spermatogenesis commonly occur. 1-4. Synizesis stage. 5-9. Preparation for spermatocyte 1. 10. Spermatocyte 1. 11, 12. Spermatocyte 2. 13. Spermatids. 14, etc. Spermatozoa in different stages of differentiation.

THE REPRODUCTIVE ORGANS.

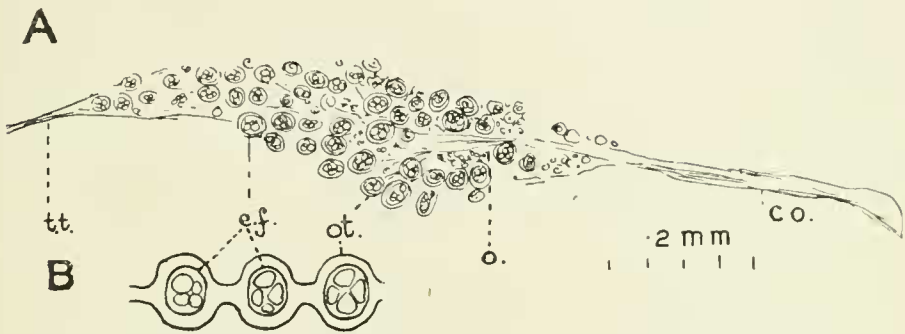
The post-embryonic development of *Culex* has been worked out by Hurst (1). The testes are paired, cylindrical in shape, and possess no receptaculæ seminales, the ripe spermatozoa being stored in the spermiducts.

The ovaries (Text-figs. 2 and 3) are paired and cylindrical, each consisting of large numbers of ovarian tubes which all open into a single duct. The two ducts, one from each side, join to form a common oviduct into which three spermathecae open.

¹ In a note appended to her paper, however, she expresses a fear that two species were used for her research.

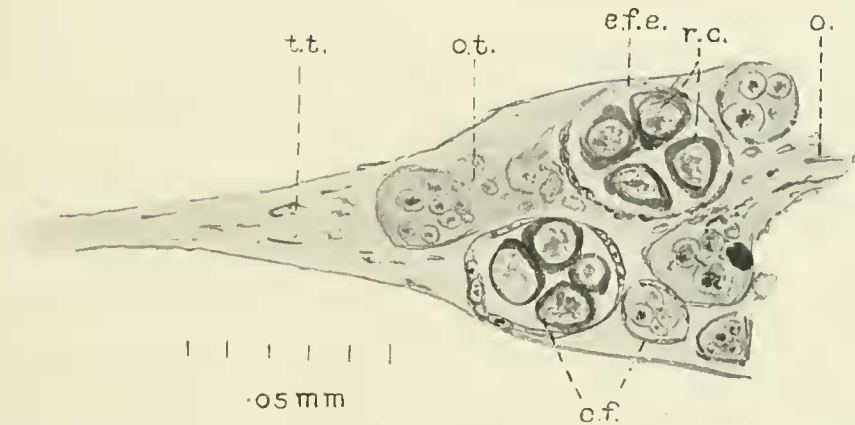
The gonad proper is contained in the third segment from the hind end of the larva or pupa, which also is true for *C. pungens* (Stevens (2)).

TEXT-FIG. 2.



A. Section through ovary of *Culex pipiens*. *c.o.* Common oviduct. *e.f.* Egg-follicle. *o.* Oviduct of right ovary. *o.t.* Ovarian tube. *t.t.* Tracheal tube. B. Diagram of ovarian tube with egg-follicles.

TEXT-FIG. 3.



Section through ovary of *Culex pipiens*. *e.f.* Egg-follicle. *e.f.e.* Egg-follicle epithelium. *o.* Oviduct. *o.t.* Ovarian tube. *r.c.* Reproductive cells. *t.t.* Tracheal tube.

SPERMATOGENESIS.

A figure of a pupal testis drawn from a reconstruction is given in Text-fig. 1. It will be seen that the testis is divided up into a number of small compartments by walls roughly at right angles to the long axis of the organ. These cysts contain cells in different stages of development, the contents

of one cyst being presumably of the same age. In this particular testis the posterior cysts are full of ripe spermatozoa; higher up, the cysts contain spermatids; next to these are cysts with immature spermatids, while higher up the compartments are full of interkinetic nuclei. Higher up still are cells, which, by their more anterior position in the gonad, and by their larger size, are presumably spermatocyte I, while at the head of the gonad are nuclei in synizesis. Thus the topographical relations of the cysts afford a means of identifying the different stages in the spermatogenesis.

After consulting many sections of larvæ and pupæ, and carefully studying the topographical relations of the cysts, it has been possible to distinguish the usual spermatogenesis stages, and this having been done, the recognition of the different stages that occur in the development of each cell generation was not a difficult matter. Although the contents of one cyst are presumably of the same age, it is often possible to find in it extremes of any stage. Thus in a cyst characterised by telophases of the first spermatocyte division there may be a few anaphases, and some metaphases, and possibly a few prophases. Working on this principle a cyst full of early prophases will sometimes contain cells undergoing preparation for prophases, and thus by linking up the information gleaned by a study of these individual variations a full series of stages can be obtained. Stress has been laid on the topographical relations of the cysts for reasons that will be apparent later.

A comparison of Text-fig. 1 with fig. 5 in Stevens' paper (2) will show that in the case of *Culex pungens* any one gonad contains a greater range of spermatogenesis stages than is the case with *C. pipiens*. Only rarely in the latter case are spermatogonial divisions found in the pupæ and old larvæ, these divisions taking place in younger larvæ. The usual distribution of stages in old larvæ and pupæ of *C. pipiens* is from synizesis to spermatozoa.

Resting nuclei in the testes of young larvæ resemble the nuclei of the connective tissue (Pl. 27, figs. 1, 2 and 3). In

the densely staining nuclear sap the chromatin is more or less peripherally arranged except for a central mass (Pl. 27, fig. 1). Resting nuclei are not found in old larvæ and pupæ—the synzesis stage apparently replacing the resting stage—nor are they very common even in the young, as there is so much active growth. In Pl. 27, fig. 3, a cluster of resting nuclei is shown from a section of a larva stained in thionin. The highly staining capacity of the cytoplasm at this stage renders the nuclei less conspicuous. Better differentiation is obtained in iron-hæmatoxylin (Pl. 27, fig. 2). In the more posterior parts of the testis of young larvæ the cytoplasm of one cell is more or less clearly marked off from that of its neighbours, but at the head of the gonad the cytoplasm, densely provided with metabolic products, forms a kind of syncytium in which large numbers of small nuclei are indiscriminately scattered. It is important to notice that there are no large dark-staining bodies in the cytoplasm, which at this stage is uniform, these dark bodies being confined to older larvæ and pupæ.

Synzesis.—The telophases of the last spermatogonial divisions are particularly interesting, because they show how the synzesis nucleus has been formed, and, as the synzesis nucleus marks the commencement of preparation for the first meiotic divisions, the whole history of the nuclear changes from the last spermatogonial division to the formation of the spermatid can be traced.

A figure of one such telophase is given on Pl. 27, fig. 4. The two daughter chromatin masses are still connected by the remains of the spindle, and round about these masses is a clear nuclear sap. The nuclear membrane is still present, this persistence of the membrane being characteristic of *Culex* mitosis as it is of that of many other insects. Each potential daughter-nucleus is possessed of a fairly thin rim of cytoplasm in which are embedded dark-staining bodies. The final separation of these two constituents would result in the formation of two nuclei, each like that drawn in Pl. 27, fig. 5—i. e. the synzesis nucleus. In this nucleus a voluminous and unstainable nuclear sap surrounds a “coagulum”

consisting of chromatin and of a substance which is apparently derived from the spindle apparatus.

This "coagulum" is frequently eccentric (Pl. 27, fig. 6). The cytoplasm of the synizesis nucleus, as was foreshadowed in the telophase of the mother-nucleus, is confined to a narrow rim. Certain dark-staining bodies differentiated by prolonged staining in iron-hæmatoxylin are to be found closely apposed to the nuclear membrane, the rest of the cytoplasm being more sparse. These dark-staining bodies are most readily discovered in material fixed in Benda, acetobichromate, and Flemming, and are only characteristic of the cytoplasm of the later stages of spermatogenesis, that of earlier stages being much more uniform, as has already been explained. Pl. 27, fig. 7, shows a cell somewhat older than that given in fig. 5, in which the dark mass of the synizesis nucleus has increased in size, the chromatin being now arranged more or less peripherally around a plasmosome, which stains very palely in the thionin sections, the chromatin being difficultly stainable except after prolonged treatment with iron-hæmatoxylin. In this latter stain the chromatin is seen to form a fairly dense crown of closely matted fine threads around a central space in which lies the plasmosome.

The densely matted masses of chromatin around the plasmosome now become disentangled to a great extent and occupy more space (Pl. 27, figs. 8 and 9). The underlying nuclear sap does not stain so deeply, so that the chromatin is more conspicuous. The plasmosome stains more deeply in thionin than it did in the stage represented by fig. 7. Fig. 8 represents a section through, and fig. 9 an uncut nucleus of this stage. In the latter figure the chromatin threads are shorter and thicker than in fig. 8, in which the nucleus is slightly younger. The cytoplasm of the cell is increasing and is filled with small granules. It has a high staining capacity.

Synizesis has now broken up; the chromatin threads, which have become thicker, lie against the nuclear membrane (Pl. 27, figs. 10 and 11). In only a few cases is it possible to count them, as they are so long and convoluted. Frequently

the apices of the loops thicken and stain deeply. The plasmosome is a highly staining and conspicuous structure.

Very often (Pl. 27, fig. 12) one chromosome thickens up before the others, which are still long and zig-zag, and not easily counted, or one part of the chromosome becomes locally thickened. This stage occurs in cysts along with nuclei containing fully formed chromosomes in late prophase. The threads frequently show a double character.

Late Prophase (Pl. 27, figs. 13 and 14).

The chromosomes have now condensed sufficiently to make the investigation of their number easy. In every case it is possible to count three chromosomes, which are sometimes double, as is often the case in meiotic prophases. The round nucleus is surrounded by a much greater quantity of cytoplasm than formerly. The chromosomes present great variety of shape. They are rod-shaped, club-shaped, dumb-bell-shaped, while crosses and rings are of frequent occurrence, these latter being formed by the partial separation of the chromosomes preparatory to metaphase. This preparation for metaphase is very often evident in the twisted character of the chromosomes, three pairs of twisted chromosomes frequently appearing (compare Stevens' fig. 14).

Metaphase (Pl. 27, figs. 15-18).

The cell itself becomes spindle-shaped. This characteristic change of shape affords help in distinguishing meiotic from spermatogonial divisions, the metaphases and anaphases of the latter taking place in round-shaped cells, the shape of the cell being in no wise affected by the formation of the spindle. The spindle shape is well shown in figs. 15-18.

A typical anaphase is illustrated by Pl. 27, fig. 19. Two of the chromosomes have each almost separated into daughter halves, the two daughter halves being merely united end to end. The third chromosome is not completely shown in the figure, part of it having been cut away.

Telophase (Pl. 27, fig. 20).

The cell now becomes greatly elongated and the chromosomes massed together. Spaces become apparent in the daughter chromatin masses, as shown in the left-hand mass in fig. 20. In the next stage these clear spaces have increased to such an extent that the central mass of the nucleus is free from chromatin, the latter occupying a peripheral position against the nuclear membrane (Pl. 27, fig. 21). It is interesting to compare what takes place here with what happened in the formation of the synzesis nucleus. In the latter case (Pl. 27, fig. 4) the vacuoles appeared round the daughter-mass of chromatin, converting this into the synzesis nucleus. In this case, however, the vacuoles appearing in the daughter mass, the chromatin is squeezed against the nuclear membrane (Pl. 27, fig. 21).

The cytoplasm in the cells, shown in figs. 12-20, is very conspicuous by its bulk, and in specimens fixed in Gilson-Petrunkewitch and Flemming (under certain conditions) it is homogeneous and deeply stainable in thionin. In some cells, notably those fixed in Benda, the cytoplasm is more like that of those cells represented in figs. 49-52, where it appears to be sharply differentiated into a more or less fluid substance and a few darkly stainable bodies.

Second Meiotic Division.

As has already been explained, no reticular resting stage follows the telophase of the first meiotic division. Vacuoles appearing in the daughter chromatin masses push the chromatin against the nuclear membrane rendering it almost invisible (Pl. 27, fig. 21). Next a plasmosome, most readily demonstrated in sections stained in Ehrlich, appears, and the chromatin thickens somewhat (fig. 22). Later (fig. 23) a "clock-face" stage similar to that which occurs in the development of the primary spermatocyte results. The chromosomes then thicken (fig. 24), though they are still in contact with

the nuclear membrane. Finally (fig. 25) three chromosomes appear in the prophase. Crosses and rings are again present, formed by the precocious longitudinal split, together with a divergence of the daughter halves. Often the chromosomes are so thick and close together that it is not easy to count them (figs. 26 and 27).

In metaphase the spermatocyte II cells are again spindle-shaped (figs. 28 and 29), but they are roughly only two thirds the linear dimension of the primary spermatocytes, and, as already explained, their position in the gonad, irrespective of their size, would render their identification an easy matter. Fig. 30 shows an anaphase of this division.

Formation of Spermatids (figs. 31 and 32).

In the round daughter-nuclei which result from the second meiotic divisions the chromatin is again to be found against the nuclear membrane (fig. 31). The nucleus now becomes elongated, the chromatin, being still peripheral, gradually diminishes in size, and finally assumes a rod shape.

Before going on to discuss the spermatogonial divisions, it may be well, in view of the difficulty of recognising the spermatogonial cells, to give some account of the nuclei in the undifferentiated gonads of young larvæ.

In the following account whenever the sex is stated it must be remembered that it is only tentatively given. The presence of cyst walls in the gonad has been the criterion for tentatively assigning the sex in the case of the male larvæ.

The ovary is very richly provided with tracheal tubes, and at a very early stage it is possible to identify these tubes. The somatic cells forming the walls of the ovarian tubes can also be distinguished from the germ cells at a very early stage. By means of these criteria it is often possible to identify as an ovary the gonad of a very young larva. However, in many cases it is not possible to say on which side lies the balance of probability.

The general facts of mitosis in very young larvæ are illus-

trated by Pl. 27, figs. 33–40. From a study of these figures it will be seen that the number of chromosomes in undifferentiated gonads, as well as in those of young male and female larvæ, is 3.

Synzesis nuclei occur in very early stages of both male and female (figs. 33 and 40). The two telophases illustrated in figs. 37 and 38 do not suggest that the daughter-nuclei will pass immediately into the synzesis nuclei (cf. fig. 4). They, therefore, belong to early generations, spermatogonial.

The effects of aceto-bichromate fixation are illustrated in figs. 39 and 40, where various metabolic constituents can be discovered in the cytoplasm (cf. figs. 53, 56, and 58).

Spermatogonial Mitosis in Young Larvæ.

Mitotic divisions in the fully differentiated, though immature, gonad of fairly young larvæ will now be discussed. In such larvæ the hinder ends of the gonad contain the more advanced stages of spermatogenesis, the degree of differentiation of spermatozoa, of course, depending on the age of the larvæ—but the anterior parts of the testis present a great difference in appearance from that of old larvæ and pupæ. Conspicuous in such gonads is the absence of synzesis stages. The cellular elements are much smaller—the cytoplasm forming a syncytium, in which the small nuclei are embedded. This, the “multiplication” stage of spermatogenesis, seems to be confined to young larvæ.

A series of division stages taken from this “multiplication” zone is given, the number of chromosomes being three (Pl. 27, figs. 41–51, and figs. 2 and 3).

The main point which emerges from a study of these nuclei is that the diploid number of chromosomes cannot be demonstrated in *Culex pipiens*. One cannot, therefore, use the number of chromosomes to distinguish spermatogonial from spermatocyte divisions. Still, a careful study of all the facts seems to show that the occurrence of a synzesis stage marks off the earlier divisions from those of spermatocyte I. The

divisions that occur in young larvæ are spermatogonial divisions, although they possess the haploid number. With regard to those synzesis stages in probable male larvæ, it must be remembered that some cells in quite young specimens differentiate very quickly. Hence the fact that synzesis stages do occur in such young creatures does not argue against the statement that the occurrence of a synzesis stage marks off spermatogonial nuclei from spermatocyte I.

OOGENESIS.

As already stated in the introduction, a full history of the facts of oogenesis can only be given when the necessary material has been collected. However, for present purposes it is only necessary to figure a few stages in order to show that the number of chromosomes in the germ-cells is three.

Three chromosomes in prophase are shown in Pl. 27, fig. 52, taken from a young larva before the "rosettes" are well differentiated. The cell in which they occur clearly belongs to an early generation.

A prophase drawn from a section of an imago, ten days old, is shown in Pl. 27, fig. 53. The cell is from a young egg-follicle, and as it is stained in thionin after fixation in Gilson-Petrunkewitch, the cytoplasm is very densely stained.

A metaphase showing six chromosomes is given in Pl. 27, fig. 54.

The drawing in Pl. 27, fig. 55 is taken from an old larva. It shows a prophase where the number of chromosomes is three. The cytoplasm is abundant.

Pl. 27, fig. 56, taken from an egg-follicle of a captive-reared imago, illustrates the oldest stage obtained. The nucleus, with its deep-staining plasmosome, and its fine, well-distributed reticulum of chromatin, forms a striking contrast to the deep-staining, voluminous cytoplasm that surrounds it.

SOMATIC MITOSIS.

Although there is much indirect evidence to show that the divisions described as spermatogonial are really divisions of

the earlier generations, in spite of the haploid and not diploid number of chromosomes, yet, in view of the possibility that they might be precocious spermatocyte I divisions, it has been thought advisable to work out the somatic mitosis of *C. pipiens* very thoroughly.

Mitosis in the Somatic Tissues of the Ovary (Text-figs. 2 and 3).

The somatic cells of the ovarian tubes of the ovary can be distinguished from the reproductive cells in very young larvæ by their minute size. The developing ovarian tubes look like rosettes in sections cut from older larvæ, the large central cells of the rosette being reproductive cells, the peripheral cells, much smaller in size, being somatic. Later on groups of from four to eight of these reproductive cells become enclosed in an epithelium—the egg-follicle epithelium—also somatic in character. The number of these egg-follicles increases with the age of the creature, the ovary having assumed its definitive arrangement in late pupal life. The exceedingly thin walls of the ovarian tubes, the nuclei of which are very small, are greatly distended by the large egg-follicles, which are made up of one egg-cell surrounded by a varying number of nurse-cells.

The ultimate fate of egg- and nurse-cells has still to be worked out. The somatic tissue of the ovary—whether the epithelium of the tubes, or the egg-follicle epithelium—is a prolific source of mitotic figures. In all cases the number of chromosomes is three.

A cell belonging to an egg-follicle in telophase is shown in Pl. 27, fig. 57, and near it is shown a reproductive cell (fig. 58), which also brings out the difference in size between the somatic and reproductive cell.

Pl. 27, figs. 59 and 60, also show three chromosomes in typically somatic anaphase.

Innumerable figures of prophases could be drawn from the preparations, it being characteristic of *Culex pipiens* that

metaphases and anaphases are comparatively difficult to find, prophases being much more abundant.

In the case of all the cells of the ovary the number of the chromosomes is three.

Somatic Mitosis (General).

Somatic mitoses, apart from those in the ovarian tissue, are by no means easily found. They do not seem to be confined to any particular period of larval or pupal life, or to take place at any fixed hour of the day or night.

The process, as observed in nerve-cells, is illustrated by Pl. 27, figs. 61-65, and Pl. 28, figs. 66-68.

It will be seen from these figures that there is a great resemblance between the reproductive cells, at certain stages of development, and the somatic cells in the nerve ganglia.

Similar likenesses between the body-wall cells and those of the gonad could also be demonstrated. Thus a very well-marked synzesis stage is characteristic, not only of the later stages of spermatogenesis and of the reproductive cells of the ovary, but also of the cells of the nervous system, and the gradual breaking up of the synzesis resembles, in the main, that which takes place in the gonad. Hence it would appear that synzesis has not the significance in the spermatogenesis of *Culex* that it is commonly believed to have in other creatures, since this phenomenon is not confined to reproductive cells.

An investigation of the tracheal tube-cells shows that the number of chromosomes is three. Very frequently the daughter halves of the split chromosome can be seen in late prophase (Pl. 28, figs. 69-71).

Evidence as to the number of chromosomes derived from a study of the body-wall cells (illustrated by Pl. 28, figs. 72-79) confirms the results already obtained elsewhere, while in the undifferentiated somatic cells in larvæ just hatched (Pl. 28, figs. 80 and 81) the number is again three.

The alimentary canal wall splits into two layers during

pupal life, the inner layer undergoing disintegration (Hurst (1)). Some of the mitoses that occur in connection with this process are illustrated in Pl. 28, figs. 82-89. The number of chromosomes is three, and in some cases the precocious tendency of the chromosomes to divide in prophase for metaphase is again evident.

Mitosis in Malpighian tubule cells is figured in Pl. 28, figs. 90-95, while fig. 96 shows an equatorial plate from a muscle-forming cell. In all cases the somatic number of chromosomes is three.

DISCUSSION.

In *C. pipiens*, as has been shown, there appear to be two maturation divisions, though no reduction of chromosomes can be demonstrated. This is contrary to general experience, for, as is well known, when, in any organism, the ripe germ-cell has the same number of chromosomes as the somatic tissues, one of the meiotic divisions is commonly omitted. It must be remembered, however, that while two divisions undoubtedly follow the synizesis stage in *C. pipiens*, the fact that they follow synizesis is the only one which has led to their separation off from the apparently similar earlier divisions, and to their being described as meiotic rather than spermatogonial. However, as nuclei closely resembling synizetic nuclei can be found in the somatic tissues of this creature, it is, therefore, possible that the synizesis nuclei in the testis have no real value in diagnosing the beginning of the meiotic phase, and that they merely represent a stage of inactivity. The rapid divisions in the "multiplication" zone result in the formation of large numbers of nuclei which, while they are awaiting differentiation into spermatozoa, remain as synizesis nuclei. When the time comes for this differentiation the synizesis nucleus begins to be active, and the stages in this awakening are analogous to the formation of spermatocyte I cells.

From the foregoing account, it will be seen that the parasynidesis for each cell generation which Miss Stevens described for *C. pungens* cannot be demonstrated for *C. pipiens*.

No figures indicating the presence of six chromosomes are to be found which cannot readily be interpreted as three chromosomes precociously split for metaphase. For example, conditions like those Miss Stevens gives in her figs. 1 and 2 (oogonia showing three pairs of chromosomes on equatorial plate) and in figs. 8, 9, and 10 (spermatogonial cells showing all three pairs in late prophase), in the light of other evidence, must, when they occur in *C. pipiens*, be described as three chromosomes already divided for metaphase. The conditions of *C. pungens*, however, offer a suggestion as to how the haploid number of chromosomes in *C. pipiens* may have been derived. The permanent fusion of the paternal and maternal members of the pair, i. e. the conversion of parasyn-desis into actual fusion, would result in the formation of three out of six chromosomes.

Miss Stevens states that in *C. pungens* the intimate relationship of the two conjugants persists from one cell generation to the next, the pairing taking place in telophase, and persisting until the metaphase of the next mitosis. From this it would seem that the conjugating chromosomes are only "unfused" in metaphase. In the case of *C. pipiens* the pairs are fused throughout the whole mitosis, hence the haploid number.

On the other hand, it is quite possible to give a different interpretation of Miss Stevens' figures of parasyn-desis from the one she offers. It is significant to note that she gives no figures in support of her statement that each of the six chromosomes (i. e. each member of the three pairs of conjugating chromosomes) found in the oogonial and spermatogonial generations divides longitudinally. She merely states the fact that they do so. Unless this division can be demonstrated, it would seem as though the so-called conjugating chromosomes were merely the daughter-halves of a precociously split chromosome, as is the case in *C. pipiens*.

An alternative suggestion, therefore, as to the chromosome complex of *Culex pipiens* and *pungens* is, that the somatic number is the same as that of the mature gamete, being three

in each case. This alternative would seem to involve the non-participation of one of the gametic nuclei in development.

Whether this is the case, or whether the homologous chromosomes are temporally united in each cell-generation of *C. pungens*, and permanently so in *C. pipiens*, can only be settled by an examination of the process of fertilisation, which I hope to undertake in the near future.

I am greatly indebted to Dr. Agar for much valuable criticism; to many friends who have assisted me in collecting material; to Mr. P. Jamieson for cutting the more important sections; and to Professor Graham Kerr for his sympathetic encouragement.

SUMMARY.

(1) The somatic number of chromosomes is three, both in the male and female of *Culex pipiens*.

(2) The number of chromosomes in the spermatogonia as well as in the primary and secondary spermatocytes and spermatids is three.

(3) The spermatogonial cells are not characterised by a synzysis stage, which latter stage marks off the spermatogonial from the spermatocyte I stage.

(4) The nuclear membrane persists throughout mitosis.

(5) The synzysis stage represents an inactive phase of the nucleus in spermatogenesis.

(6) A synzysis stage occurs in somatic nuclei.

LITERATURE.

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